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**14. ABSTRACT**
Epidemiological observations indicate that breast cancer risk is lower in visually impaired women compared to sighted women and that risk is inversely correlated with degree of visual impairment. A hypothesis to explain these findings is that blind people are less susceptible to suppression of melatonin by light exposure at night and therefore have higher levels of melatonin. Melatonin has oncostatic properties in vitro. In a survey of 12,000 blind women, we will test the hypothesis that 1) the distribution of known reproductive risk factors for breast cancer among blind women will be consistent with lower risk when compared to the general population. In a subset of 240 women, we will test the hypotheses that 2) urinary melatonin levels are lower and estrogen levels are higher among blind women with light perception compared to women without light perception; 3) melatonin levels will be higher and estrogen levels lower among totally blind women who have non-24-hour melatonin rhythms and therefore a confirmed absence of light-induced suppression of melatonin, compared to totally blind women who have 24-hour melatonin rhythms and may be affected by light. Data collection is ongoing and there are no results to report at this time.

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## INTRODUCTION

Since 1940, breast cancer incidence rates have been steadily rising in the United States (1). There is growing evidence for possible effects of exposure to light at night (LAN) on cancer risk due to the increased use of modern electric lighting (2-8). Epidemiological observations indicate that breast cancer risk is lower in women who are visually impaired as compared to the sighted population and that the risk may be inversely correlated with degree of visual impairment (9-13). One hypothesis proposed to explain these findings is that blind people are less susceptible to suppression of melatonin by light exposure at night and therefore have higher circulating levels of melatonin. Melatonin has been shown to have oncostatic properties in vitro (14). Frequent light-induced melatonin suppression has been hypothesized as a cause of the higher breast cancer incidence observed in female shiftworkers and flight-attendants (3-6,15-17). Blindness is also associated with disorders of the circadian system (18) and changes in reproductive function (19-20) which may also contribute to breast cancer risk. The aim of this study is to investigate further the relationship between the severity of blindness and melatonin and estrogen production while simultaneously assessing how blindness and/or melatonin production are related to known risk factors for breast cancer.

## BODY

The study design and approved Statement Of Work is divided into two parts; Part 1 is an epidemiological health survey of breast cancer risk in 12,000 blind women and Part 2 is an assessment of melatonin and estrogen Levels in a subset of 240 blind women.

### Statement of Work progress report

Part 1 – Epidemiological Survey of Cancer in the Visually Impaired

*Task 1 (Months 1-4).* All elements of Task 1 have been completed as described in the previous report.

*Task 2 (Months 5-12).* Task 2 has been completed as described in the previous report.

*Task 3 (Months 12-24).*

a) and b) Recruitment for the epidemiological survey is ongoing and additional advertisements and study updates have been prepared for publication in 'Braille Forum' [publication of the American Council of the Blind (ACB)] and 'Braille Monitor' [publication of the National Federation of the Blind [NFB]. We were also very active at the annual conventions of both the ACB and NFB in July 2005 to try to raise awareness of the study including invited convention seminars, study workshops and an exhibition stand at each meeting. We will attend the meetings again in 2007 to disseminate the findings of the study.

In addition to recruitment via general advertisement, we have pursued the method of individual mailshots to members of several associations which traditionally have a higher return rate than general advertisements. Firstly, in collaboration with the Perkins Braille and Talking Book Library, Boston we sent an informational letter about the study to 8,000 female library borrowers. We had a response rate of 5-10%, compared to ~1% for advertising in general publications. The demographic of this group was quite elderly and we anticipate greater response rates in future mailshots that target a younger population. To this end, the ACB have agreed to allow mailshots of their ~25,000 female members nationwide. We are currently preparing our recruitment information in the range of media required (large print, Braille, audiotape) and anticipate sending out the first mailing in June 2006. We are also currently in negotiations to make a similar arrangement with the NFB to mail its ~40,000 female members. We hope that these initiatives will increase our study population substantially. Additional advertising has been sent to more than 150 radio reading services for the visually impaired nationwide, resulting in several radio interviews, and several hundred associations for the visually impaired. We have also posted the study on many listservs and user groups online as well links to

many web-sites for the visually impaired. We will continue these efforts to further expand our study population.

c) As a result of our advertising efforts, we have completed surveys in more than 1000 visually impaired women to date and have therefore developed the largest database of concurrent information on breast cancer risk factors in visually impaired women worldwide. Preliminary findings from the first 515 women are reviewed below.

d) We have not yet contacted any subjects to complete the survey a second time as part of the prospective study. We are focusing on expanding the initial database as much as possible given that we have not recruited as many women as initially proposed. A prospective cohort will established from the initial subject population in months 25-30.

e) As outlined in the previous annual report, we have developed a web-based data entry tool and database structure that permits automatic coding of the data during the data export process. We have made some modifications to the coding of some free-text entries, particularly the diagnosis of visual impairment. A drop-down menu has been added to the web-based tool for the 10 most prevalent causes of blindness to aid coding.

Part 2 – Assessment of Melatonin and Estradiol Levels in the Visually Impaired

*Task 1 (Months 1-4).* All elements of Task 1 have been completed as described in the previous report.

*Task 2 (Months 5-12).* Task 2 has been completed. a-g) We have initiated part 2 of the study with 82 subjects to date and sleep log and available urine data for these subjects have been entered into our database. A; h) Urinary 6-sulphatoxymelatonin assays have been completed for 45 subjects, and data from the first 22 are reported below. Urinary 6-sulphatoxymelatonin measurement for the remaining subjects and estrone-3-glucuronide assays for all samples are underway.

*Task 3 (Months 13-36).* Task 3 is ongoing. a-f) In addition to the 82 subjects who have already completed the 8-week field study, and additional 28 women have indicated an interest in participating and currently undergoing screening and recruitment. We expect that many more will volunteer as the epidemiological survey numbers increase. With the help available through our summer student program (see below), we intend to initiate field studies of 40 subjects per month for the next four months and therefore anticipate completing all 240 subjects by the fall of 2006; g) Data entry will be completed as data are returned and therefore we anticipate that all field study data entry will be completed by the end of 2006; h) Urinary assays will be scheduled in batches of~50 subjects in order to reduce interassay variability and sent for processing as soon as each batch is ready. The laboratory turnaround for each batch is ~3-4 weeks therefore we anticipate having all assay results completed by the end of 2006.

*Task 4 (Months 12-36).* Task 4 is ongoing. a-d) Data entry, quality control and data plotting for the sleep logs and urinary 6-sulphatoxymelatonin rhythms have been completed for the subjects studied to date. The data for the first 22 subjects are reported below. As outlines for Task 3, we anticipate that all data collection will be completed in the fall of 2006, and that all quality control, data entry, plotting and identification of circadian rhythms disorders will be completed by December 2006. e-f) We intend to complete the primary statistical analyses and write the final report from Jan-May 2007, as scheduled.

**Research findings for the period of the report**

Part 1 – Epidemiological Survey of Cancer in the Visually Impaired

Methods

The primary reporting tool for this cross-sectional study was an epidemiological survey (see Appendix A in annual report 2005). The survey consisted of 126 questions about known breast cancer risk factors ranging from reproductive risk factors (e.g. age of menarche, menopause, first childbirth,

lactation) to factors such as diet, alcohol use, body habitus, exercise and family history. The survey also contained questions about visual impairment including diagnosis, current eye conditions, visual acuity, degree of light perception and age of onset of visual impairment. The survey included the Harvard National Depression Screening Scale (HANDS) and socioeconomic questions. Finally, the survey included the Pittsburgh Sleep Quality Index (PSQI) and additional sleep questions to determine the presence of a circadian sleep disorder.

The survey was provided in a variety of formats including Braille, large print, audio, via the internet, e-mail or verbally over the telephone.The web-based survey was developed by Velir studios and is compliant with US section 508 (Americans with Disabilities) guidelines for web pages. The survey was also rigorously tested by over 20 blind computer users with varying degrees of vision, computer skills and assistive technologies. The web-based survey also served as a study database and surveys completed in other formats were entered by research staff. The survey was compliant with Health Insurance Portability and Accountability Act (HIPAA) privacy standards by separating identifiable information from survey information on two separate servers. Ethical permission for the study was granted from the Institutional Review Board at Brigham and Women's Hospital and the United States Department of Defense Human Subjects Research Review Board. Informed consent was obtained from all subjects.

Data comprising the present analysis was exported on March 19, 2006. Test survey entries, duplicate subject survey entries, data from subjects who completed <30% of the survey and data from male participants were removed from the analysis. Data was sorted and answers open ended survey questions were coded to correct misspellings and ensure consistency between responses. For example, all subjects reporting 'retrolental fibroplasia' as the primary eye condition were recoded to the 'retinopathy of prematurity' group. Descriptive statistics were compiled using Intercooled Stata Version 8.2 software (StataCorp LP, College Station, Texas, USA). No formal interim statistical analysis has been performed on the epidemiological survey data in order to avoid the need for adjusting the statistical probability in the final primary analyses.

## Results

### *Visual impairment*

Two hundred ninety six subjects reported some degree of light perception (LP), while 211 reported being unable to perceive light (NPL) and eight did not report degree of light perception. Five of the NPL subjects did not answer the question about degree of light perception, but reported removal of both eyes and were therefore classified as NPL. LP subjects were further classified by their reported degree of light perception from corrected vision in the better eye as; able to see the top letter on the vision chart (n=113), able to count fingers (n=53), able to see shadows and hand movement (n=58), able to see light (n=72). NPL subjects were also been further classified by number of eyes enucleated; 122 reported both eyes present, 16 reported one eye enucleated, 69 reported both eyes enucleated and four subjects did not answer the question about enucleation. Subjects were also classified according to type of ocular disorder (see Table 4).

### *Age and BMI*

The mean age of the entire cohort was 49.23 (±14.76) and mean age increased with respect to menopausal status (Table 1). The BMI for the entire cohort was overweight at 29.0, with the average weight for the cohort as 168.69 (±45.52) pounds and the average height was approximately 5 ft. 3 in. (±2.89 in.). The BMI for all menopausal categories fell within the definition of borderline obesity, which is a BMI score of ≥30 (Table 1).

### *Sleep Disorders*

The mean PSQI score (range 0-21, with a score of 5 indicating a sleep disorder) increased with age and menopausal status and was elevated in all groups, with a cohort mean of 8.11 (± 4.33) (Table 1).

**Table 1.** Demographic information and PSQI scores for subjects sorted by menopausal status.

| Menopausal Status | N | Age (n) | | Weight (n) | | Height (n) | | BMI | LP/NPL/ Unreported | PSQI Score (n) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Pre-menopausal | 196 | 35.81 (194) | ±9.73 | 164.87 (195) | ±46.96 | 63.69 (196) | ±3.02 | 28.3 | 133/63/0 | 7.53 (176) | ±4.09 |
| Peri-menopausal | 64 | 51.67 (64) | ±3.69 | 174.69 (63) | ±48.25 | 62.71 (62) | ±3.05 | 30.8 | 33/29/2 | 8.04 (57) | ±4.21 |
| Post-menopausal | 227 | 60.00 (224) | ±11.08 | 169.06 (227) | ±43.47 | 63.56 (223) | ±2.71 | 29.0 | 111/111/5 | 8.71 (195) | ±4.51 |
| Unreported | 28 | 50.48 (27) | ±10.58 | 178.71 (28) | ±44.61 | 64.68 (28) | ±2.53 | 29.6 | 19/8/1 | 6.9 (10) | ±4.86 |
| Entire Cohort | 515 | 49.23 (509) | ±14.76 | 168.69 (513) | ±45.52 | 63.56 (509) | ±2.89 | 29.0 | 296/211/8 | 8.11 (438) | ±4.33 |

PSQI=Pittsburg Sleep Quality Index. Score =5 indicates disordered sleep. BMI=Body Mass Index. Underweight =18.5, Normal weight = 18.5-24.9, Overweight = 25-29.9, Obesity = 30

The PSQI scores do not appear to be associated with degree of light perception for subjects who reported light perception, however NPL subjects had a higher mean PSQI score than combined LP subjects, although they were also slightly older (Table 2). Subjects who had both eyes removed had the highest mean PSQI score (8.79 ±4.81) as compared with those who had one or no eyes removed (Table 3). The PSQI score appears to vary by the type of ocular damage with those subjects suffering from primarily lens of pigment epithelium disorders (cataracts, retinitis pigmentosa) having a lower severity of sleep disorders compared to diseases that affect the optic nerve or ganglion cell layer (glaucoma)(Table 4).

| Level of Vision | N | PSQI Score | | Age (n) | |
|---|---|---|---|---|---|
| Eye Chart | 89 | 8.17 | ±4.36 | 47.26 (89) | ±14.10 |
| Counting Fingers | 50 | 7.24 | ±3.74 | 51.9 (50) | ±19.21 |
| Shadows/Hand Movement | 53 | 8.28 | ±4.39 | 43.77 (52) | ±15.54 |
| Light Perception Only | 61 | 7.89 | ±4.53 | 46.01 (61) | ±13.68 |
| LP | 253 | 7.94 | ±4.29 | 47.15 (252) | ±15.87 |
| NPL | 178 | 8.37 | ±4.42 | 51.08 (175) | ±13.13 |
| Unreported | 7 | 7.43 | ±3.95 | 54 (7) | ±3.74 |

**Table 2.** Mean age, PSQI score and degree of LP for all completed PSQI questionnaires, ±SD.

| Eyes Enucleated | N | PSQI Score | |
|---|---|---|---|
| None | 340 | 8.06 | ±4.28 |
| One | 34 | 7.26 | ±4.17 |
| Both | 57 | 8.79 | ±4.81 |
| Unreported | 7 | 8.71 | ±3.30 |

**Table 3.** Mean PSQI score sorted by number of eyes removed, ±SD .

| Eye Condition | PSQI Score (n) | |
|---|---|---|
| Anopthalmia/Coloboma/Micropthalmia | 6.25 (12) | ±4.31 |
| Cataracts | 7.15 (27) | ±4.04 |
| Retinitis Pigmentosa and Related Disorders | 7.48 (52) | ±4.42 |
| Retinoblastoma | 8.10 (21) | ±5.24 |
| Macular Degeneration and Related Disorders | 8.10 (30) | ±4.82 |
| Optic Nerve Related Disorders | 8.13 (30) | ±4.08 |
| Glaucoma | 8.31 (36) | ±4.33 |
| Other/Unknown/Unreported | 8.33 (73) | ±4.26 |
| Retinopathy (of prematurity and diabetic) and Retinal Detachment | 8.47 (157) | ±4.24 |

**Table 4.** Mean PSQI score sorted by eye condition for the entire cohort, ±SD.

*Reproductive function and history*

The mean age of menarche for the entire cohort was 12.24 (±1.54) and did not appear to vary with degree of light perception, though subjects reported vision loss before age 12 had a slightly earlier menarche compared to those who lost their vision after age 12. The mean age of menopause for the entire cohort was 45.75 (± 8.13) with NPL subjects reporting a slightly later menopause compared to LP subjects (Table 5). Nearly half of the cohort reported at least one pregnancy (n=244) and the average number of pregnancies was 2.55 (± 2.02) per woman who reported pregnancy. The average age at first full term pregnancy was 25.61 (± 5.49) for the entire cohort. Sixty-nine women reported a history of at least one miscarriage, 43 reported a history of at least one terminated pregnancy and 21 reported pregnancies that did not run to term for other reasons. More than half (61%) of the women who had pregnancies reported a history of breast feeding. LP subjects had more pregnancies, with an average of 2.72 (±2.34) at a younger average age 24.75 (± 5.14) compared to NPL subjects who averaged 2.32 pregnancies with an average first birth at age 26.62 (± 5.78). There were no other apparent differences between LP and NPL subjects.

Twenty percent of the total cohort reported having a hysterectomy representing 38% of the post-menopausal subjects. Fifty-one subjects in both the LP and NPL groups reported having hysterectomies. This represents 17% of the total LP subjects and 38% of the post menopausal LP subjects and 24% of the total NPL subjects and 39% of the post menopausal NPL subjects.

| | | Puberty | | | Menopause | | |
|---|---|---|---|---|---|---|---|
| **LP/NPL** | **Age of Visual Loss** | **N** | **Mean Age** | | **N** | **Mean Age** | |
| LP | Visual Loss < 12 | 188 | 12.11 | ±1.47 | 81 | 44.67 | ±8.21 |
| | Visual Loss =12 | 91 | 12.66 | ±1.67 | 54 | 45.39 | ±8.18 |
| | All | 279 | 12.29 | ±1.56 | 135 | 44.96 | ±8.17 |
| NPL | Visual Loss < 12 | 171 | 12.08 | ±1.40 | 112 | 46.21 | ±8.34 |
| | Visual Loss =12 | 31 | 12.52 | ±2.05 | 18 | 47.56 | ±7.20 |
| | All | 202 | 12.14 | ±1.52 | 130 | 46.4 | ±8.18 |
| Unreported | Visual Loss < 12 | 7 | 13.29 | ±1.38 | 7 | 49.14 | ±3.93 |
| | Visual Loss =12 | 0 | - | - | 0 | - | - |
| **Entire Cohort** | **All** | **488** | **12.24** | **±1.54** | **272** | **45.75** | **±8.13** |

**Table 5.** Mean onset of puberty (defined as age of first period) and menopause (defined as the age periods stopped completely) for the entire cohort categorized by subjects with and without light perception and further categorized by whether age of onset of visual loss occurred prior to age 12, ±SD.

*Exercise and diet*

Hours of exercise per week averaged 7.19 (± 8.66) hours per week for the group, with LP subjects averaging just over half an hour more per week (7.49 ± 8.81 h) than NPL subjects (6.87 ± 8.48 h). Caffeine consumption per day in the entire cohort averaged 3.04 cups (±3.01), with NPL subjects consuming more caffeine on average (3.13 ± 3.13) than LP subjects (2.95 ± 2.92 cups/day). Alcohol consumption averaged 1.33 glasses per week for the entire cohort, with NPL subjects consuming more alcohol on average than LP subjects (LP 1.15 ±2.63, NPL 1.64 ±3.46).

*Sleep medication use*

| | Use Sleep Aids | | | Use Nothing | | |
|---|---|---|---|---|---|---|
| | **N** | **PSQI Score (n)** | | **N** | **PSQI Score (n)** | |
| LP | 45 | 10.64 (39) | ±3.81 | 237 | 7.45 (214) | ±4.19 |
| NPL | 46 | 10.34 (38) | ±4.08 | 160 | 7.78 (138) | ±4.35 |
| Unreported | 2 | 9.00 (2) | ±2.83 | 5 | 6.8 (5) | ±4.44 |
| Entire Cohort | 93 | 10.46 (79) | ±3.90 | 402 | 7.59 (359) | ±4.26 |

**Table 6.** Mean PSQI scores categorized by light perception and sorted by use of melatonin and/or prescription sleep aids, ±SD.

Eighteen percent of the entire cohort reported using prescription sleep aids or melatonin. Twenty-one percent of NPL subjects reported using prescription sleep aids or melatonin, compared to 15% of LP subjects. Subjects who reported using melatonin or hypnotics had higher PSQI scores compared with subjects who did not (Table 6).

Part 2 – Assessment of Melatonin and Estradiol Levels in the Visually Impaired

## Methods

*Subject details*

Data from 22 subjects have been included in the present field study analysis. Of these, subjects 11 reported pre-menopausal status, two reported peri-menopausal status and nine reported post-menopausal status (Table 7). Twelve subjects reported some degree of light perception, nine reported no light perception and one subject did not report degree of light perception (Table 7) (the subject who did not report her level of light perception was questioned extensively following the survey and it seems likely that she is an NPL subject, however she was unable to distinguish whether she was able to see light or simply perceive an artefact of light such as heat, therefore this subject is classified as 'unreported' throughout the present report).

Pre-menopausal subjects were younger (43.8 ± 7.67 years) than post-menopausal subjects (56.1 ± 4.4 years) and had lower mean PSQI scores (6.11 ± 3.69 versus 8.88 ± 6.33, respectively). Post-menopausal subjects were taller and lighter than pre-menopausal subjects with a mean BMI of 25.7 compared to 28.1 for pre-menopausal subjects.

Retinopathy of Prematurity was the most common diagnosis in the field study cohort afflicting eight of the subjects (Table 7). The majority of subjects (15) reported being blind since birth of which only one subject reported progressive visual loss. Of the subjects who lost their vision after birth, four reported progressive vision loss (Table 7). Two field study subjects reported enucleation of both eyes and one subject reported enucleation of one eye.

| Subject | Eyes Removed | Age | PSQI Score | Diagnosis | LP/NPL | Age of Onset of Visual Loss | Rapidity of Loss | Menopausal Status |
|---|---|---|---|---|---|---|---|---|
| 25BC105 | . | 56 | 3 | Retinopathy of Prematurity | NPL | Birth | . | Post-menopause |
| 25BC108 | . | 29 | 13 | Leber's Congenital Amaurosis | LP | Birth | . | Pre-menopause |
| 25BC113 | . | 53 | 3 | Osteoma of the Skull | LP | 6 | instantly | Peri-menopause |
| 25BC119 | . | 33 | 2 | RetinoBlastoma | NPL | 2 | . | Pre-menopause |
| 25BC121 | both | 47 | 9 | Microopthalamia/Glaucoma | NPL | Birth | . | Pre-menopause |
| 25BC126 | . | 32 | 8 | Aniridia | LP | Birth | years | Pre-menopause |
| 25BC141 | . | 57 | 10 | Retinopathy of Prematurity | NPL | Birth | . | Post-menopause |
| 25BC149 | . | 52 | 9 | Retinopathy of Prematurity | . | Birth | . | Post-menopause |
| 25BC159 | . | 51 | Incomplete | Accident | NPL | 3 | instantly | Pre-menopause |
| 25BC167 | . | 54 | 9 | Retinopathy of Prematurity | LP | Birth | . | Post-menopause |
| 25BC177 | right | 50 | 8 | Cataracts | LP | Birth | . | Pre-menopause |
| 25BC189 | . | 54 | 1 | Glaucoma | LP | Birth | . | Post-menopause |
| 25BC207 | . | 52 | 4 | Retinopathy of Prematurity | NPL | Birth | . | Pre-menopause |
| 25BC232 | . | 57 | 18 | Kerotoconus | LP | 12 | years/instantly | Post-menopause |
| 25BC236 | . | 54 | Incomplete | Retinopathy of Prematurity | NPL | Birth | . | Post-menopause |
| 25BC239 | . | 47 | 3 | Cataracts | NPL | Birth | . | Pre-menopause |
| 25BC259 | . | 42 | 2 | Unknown | LP | Birth | . | Pre-menopause |
| 25BC292 | . | 49 | 2 | Aniridia | LP | Birth | . | Peri-menopause |
| 25BC293 | . | 41 | 7 | Retinopathy of Prematurity | LP | Birth | . | Pre-menopause |
| 25BC320 | both | 54 | 13 | Retinopathy of Prematurity | NPL | 3 | weeks | Post-menopause |
| 25BC356 | . | 46 | 5 | Stargardt'sDisease | LP | 14 | years | Pre-menopause |
| 25BC475 | . | 67 | 2 | Retinitis Pigmentosa | LP | 39 | years | Post-menopause |
| **Mean** | **-** | **49 ±9.04** | **7 ±4.87** | **-** | **12/9/1** | **-** | **-** | **11/2/9** |

Table 7. Details for 22 field study subjects.

*Sleep and urinary hormone data collection*

Subjects completed a sleep, nap and (in pre and peri-menopausal women) menstrual cycle diary for eight weeks. Subjects also wore an activity monitor continuously during the eight week period. All subjects completed two 48h sessions of urine samples. The first set of samples was collected after subjects completed the sleep diary for two to four weeks. The second set of samples was collected four to six weeks following the first set. The subjects were instructed to collect all urine over the

course of 48 hours in four hourly bins throughout the waking period and eight hourly periods throughout the sleep period. Subjects were instructed to collect urine starting after the first morning void on the first day of collection. They were instructed to weigh each sample using speaking scales at the end of each sampling window and were instructed to pipette a small sample from each window into a 7ml tube and immediately freeze each sample. Subjects were asked to record the times of each void, the sample window and the total urine volume for each sample period. Subjects were not asked to alter any lifestyle habits throughout the study.

*6-sulphatoxymelatonin (aMT6s) assay and analysis*

Urinary aMT6s concentrations were measured by Stockgrand Ltd., University of Surrey (Guildford, UK) by radioimmunoassay (RIA) using the method of Aldhous and Arendt. All samples from an individual were measured in a single assay. The interassay coefficients of variation were 12.3% for 4.3 ng/mL, 12.8% for 13.3 ng/mL and 11.3% for 26.3 ng/mL.

The mean 24h aMT6s output was calculated for each subject for each sample period. Data were grouped by degree of light perception and by age (18-29, 30-39, 40-49, 50-59, ≥60).

For analysis of the circadian rhythm of aMT6s, aMT6s data were converted into micrograms per hour for each 4-8 hourly collection period in. Data for each subject and sample period were plotted and subjected to cosinor analysis (software provided by Dr. D. S. Minors, University of Manchester, Manchester, UK) to provide the acrophase (peak) time and the amplitude of the aMT6s rhythm. Only results that showed a significant fit to the cosine curve ($P<0.05$) were used to assess entrainment. Subjects were considered normally phased if the mean of the two acrophase times fell within the normal range as described in Lockley et al., 1997 (range; 1.3-7.1)(18). Subjects were considered 'abnormally phased' if the mean of the two acrophase times fell outside the normal range.

## Results

*6-sulphatoxymelatonin 24-h production*

All 22 subjects produced measurable amounts of aMT6s. LP subjects produced slightly more 24h aMT6s than NPL subjects while LP subjects were younger on average than NPL subjects (Figure 1). When categorized by age older subjects tended to produce more aMT6s on average than younger subjects. When categorized by degree of light perception, those who reported having light perception only produced the highest mean amount of aMT6s (36.20 ±15.77, n=4) and the subject who reported being able to count fingers had the lowest aMT6s production (11.44, n=1).

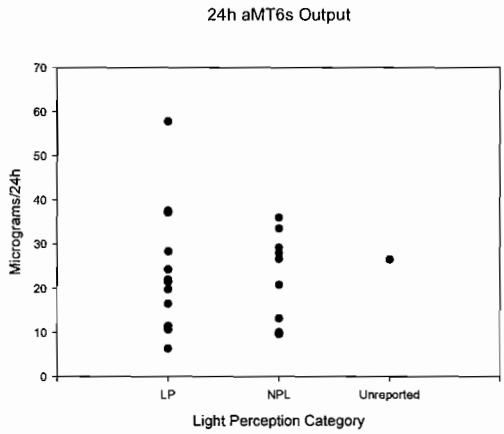
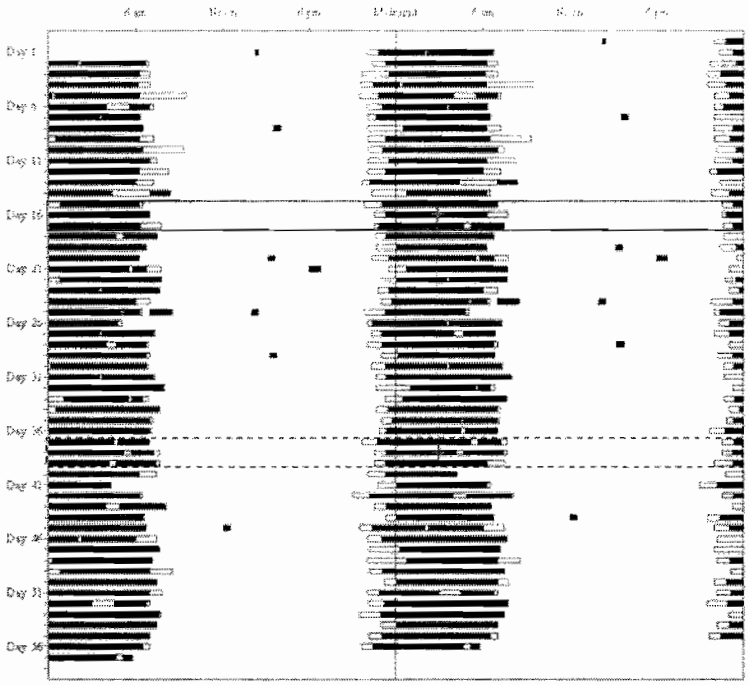



**Figure 1.** Mean 24-hour aMT6s production for 22 subjects sorted by light perception.

*6-sulphatoxymelatonin circadian rhythmicity*

When assessed by cosinor analysis, 10 of the 22 subjects included in the present analysis showed a significant aMT6s rhythm. Of these subjects, three reported some degree of light perception, six reported having no light perception and one subject did not report degree of light perception. Two of the LP subjects and one of the NPL subjects were classified as normally phased (mean acrophase within normal range 1.3-7.1). Three of the NPL subjects were classified as not abnormally phased (mean acrophase outside the normal range) and the remaining subjects could not be classified by circadian phase. Figures 2-3 show representative plots of the self-reported sleep and nap diary and results of aMT6s rhythms for 2 subjects, one with normally phase rhythms (Figure 2) and one with non-entrained circadian rhythms (Figure 3).

## Figure 2



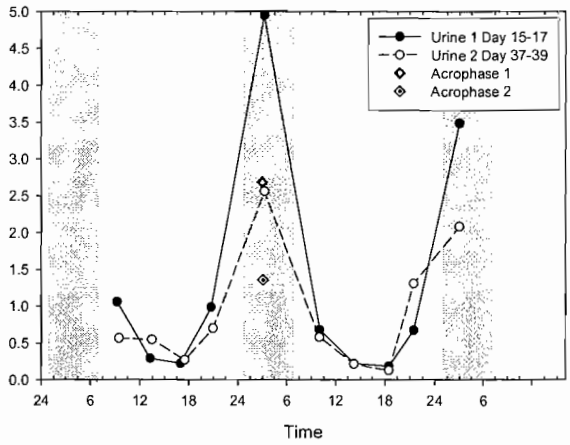
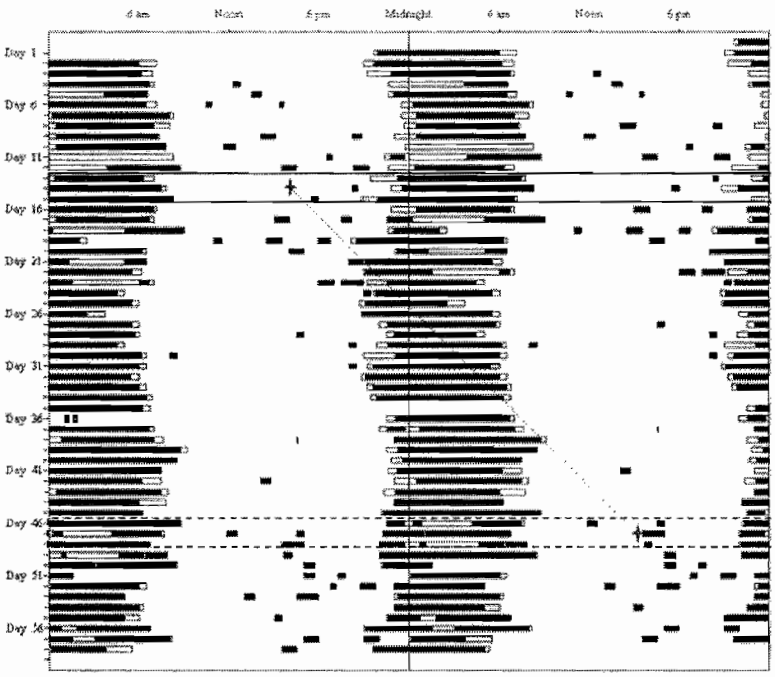



**Figure 2.** Representative raster plot and 48h aMT6s profile for a normally phased pre-menopausal LP subject. A) Raster plot of self reported sleep and nap times. Data are double plotted with clock hour across the top and day of study on the y axis. Black bars indicate sleep, open bars indicate times awake in bed. The solid black bracket encases the sampling days for the first set of urine samples and the dashed bracket encases the sampling days for the second set of urine samples. Red stars indicate aMT6s acrophase times taken from cosinor analysis. The line connecting the acrophase times is for visual orientation only. B) 48h aMT6s profile in micrograms per hour. Filled circles correspond to the first urine sample period, open circles correspond to the second urine sample period. Diamonds represent acrophase times as assessed by cosinor fit and shaded areas indicate the range of normal acrophase time.

## Figure 3



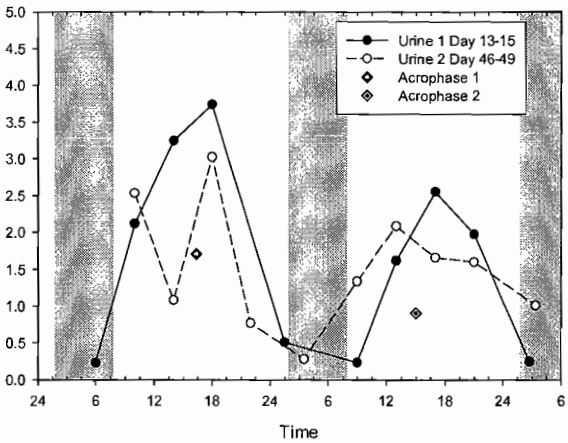



**Figure 3.** Representative raster plot and 48h aMT6s profile for an abnormally phased pre-menopausal NPL subject with a non-entrained circadian system (key as for Fig 2). Note that the peak aMT6s time is delayed during the second collection compared to the first and is accompanied by daytime napping. The characteristic cycling of episodes of good sleep (days 26-46) and bad sleep (days 1-25 and 47 to 58) indicative of non-24-hour sleep wake disorder is easily observed.

Discussion

While the current epidemiological findings from Part 1 of study are preliminary, there are several trends that may be indicative of alterations in risk factors associated with breast cancer in visually impaired women, although until formal multivariate statistical analysis has been completed, these indications should be considered observational. Firstly, the high BMI reported for the pre-menopausal cohort has been associated with a reduced risk of breast cancer (21,22), although BMI was high for the cohort as a while. Secondly, the population mean alcohol intake is also low, which is also associated with lower breast cancer risk (23), although NPL subjects drank slightly more than LP subjects. Thirdly, there may be changes in reproductive function (19,20) that are consistent with a reduced risk. While the age of menarche did not appear to differ from national averages (National Women's Health Information Centre http://www.womenshealth.gov/faq/menstru.htm, a more substantial difference was observed in the age of menopause. Our cohort had a mean menopause age of 46 years, which is younger than the average for the general US population at age 51 (National Women's Health Information Centre http://www.womenshealth.gov/faq/menstru.htm). Furthermore, 38% of the total cohort (compared to 33% nationally (24)) had had a hysterectomy (and therefore caused menopause) with an average age of 41 years. Thus, the net reduction in estrogen exposure due to earlier menopause both spontaneously and after hysterectomy, may contribute to the reported reduced risk in breast cancer among blind women.

Other preliminary findings are not consistent with reduced breast cancer risk, however. The average age of first pregnancy was 26 year compared to the average first pregnancy among US woman occurring at age 21.4 in 1970 or 24.9 in 2000 (25), which is associated with higher breast cancer risk. Subjects with light perception (LP) had more pregnancies on average at a younger age than subjects no perception of light (NPL) and history of miscarriage and breastfeeding were roughly equal for LP and NPL subjects, none of which are consistent with reduced risk.

The aim of Part 2 of the study is to assess whether light is reaching the circadian pacemaker, and therefore the pineal gland, and consequently suppress circulating levels of melatonin as an indicator of increase breast cancer risk. Firstly, in Part 1, we used the Pittsburgh Sleep Quality Index as a proxy marker for light input to the clock as blind subjects not receiving light information are highly likely to develop circadian rhythm sleep disorder as a result (26). Those with a sleep disorder who are not affected by light may therefore by hypothesized to have higher circulating levels of melatonin (18). In the current analyses, mean PSQI scores did not show a clear dose-dependent effect with degree of light perception, but did appear to show that NPL subjects had higher average PSQI scores than LP subjects, as predicted. Similarly, a higher percentage of NPL subjects (21%) reported use of sleep aids compared to LP subjects (15%) and had slightly more caffeine consumption per day suggesting increased levels of daytime sleepiness (27).

The preliminary data from part two of the study does not suggest that melatonin levels are higher in NPL compared to LP subjects (Figure 1), although only a small number of subjects have been assessed to date.

**Problems encountered in accomplishing the Statement Of Work**

We have not been able to achieve the anticipated recruitment rate to date for the epidemiological survey. We had hoped to establish a database with 12,000 participants and to date, we have reached more than 1000. While this study still represents the largest and most comprehensive database of breast cancer risk factors in the visually impaired constructed to date, we are still some way short of our goal. We have advertised to hundreds of associations for the visually impaired nationwide, including the two major associations, radio reading services, libraries, listservs, and web-sites. Recently, however, we made what we hope are two major breakthroughs in our recruiting efforts. Firstly, we sent individual mailshots to 8,000 women who were members of the Perkins Library in Boston and have received ~400 survey responses (~5%) to date, a higher proportion than our general advertising. We have taken this finding further and negotiated a similar arrangement with the American Council of the Blind (ACB) to send mailings to ~20,000 of it female members in summer 2006. We hope that this will add several thousand participants to our survey and are optimistic of a >5% recruitment rate given the younger age of the ACB members. Furthermore, we are trying to negotiate a similar arrangement with the National Federation of the Blind to send individual mailings to

its ~40,000 female members. Should these initiatives be successful, we hope to recruit several thousand more women to the study.

**Additional accomplishments**
We continue to attract high caliber students to assist with the project and, to date, we have had 19 student volunteers assist with recruitment, data collection, and data entry. In summer 2006, we will repeat our successful summer student program and anticipate hosting 4 full-time and 6 part-time students who will assist with the study. The students will assist in study advertising, subject recruitment, informed consent procedures, survey completion and identification of subjects for the field-based study. We have also prepared a seminar series to support their research efforts and the students will attend regular seminars given by leading researchers in the field of breast cancer, epidemiology and circadian biology.

## KEY RESEARCH ACCOMPLISHMENTS

- We have surveyed >1000 visually impaired women and begun to establish a comprehensive database addressing a wide range of risk factors associated with breast cancer in this population
- Preliminary analysis suggests that blind women may have changes in reproductive history and function that may reduce breast cancer risk
- We have studied >80 women under field conditions to establish whether there is a link between degree of blindness and circulating melatonin levels
- We have developed a summer undergraduate training program in circadian biology and breast cancer

## REPORTABLE OUTCOMES

Databases

As described above, we have constructed a database of more than 1000 visually impaired women for the assessment of risk factors associated with breast cancer including visual impairment, reproductive function and history, diet and circadian rhythm desynchrony.

Abstracts and Presentations

2004 Lockley SW. Circadian rhythms in blind women. Circadian Disruption and Breast Cancer Meeting; 2004 Jul 9-11; Chapel Hill.

2005 Evans EE, Schernhammer ES, Silver ES, Stevens RG, Lockley SW. Reproductive and hormonal risk factors for breast cancer in blind women. Dana-Farber/Harvard Cancer Center Cancer Disparities Program, New Investigators Poster Session; 2005; Apr 15; Boston,

2005 Lecture Series: Reproductive and hormonal risk factors for breast cancer in blind women Summer undergraduate program (10 x 3-h lectures), Division of Sleep Medicine, Brigham and Women's Hospital; 2005; Jun 1-Aug 16; Boston. [Appendix A]

2005 Evans EE, Schernhammer ES, Silver ES, Stevens RG, Lockley SW. Reproductive and hormonal risk factors for breast cancer in blind women. Era of Hope Department of Defense Breast Cancer Research Program Meeting; 2005; Jun 8-11; Philadelphia

2005 Lockley SW. Urinary aMT6s measures for epidemiological studies. Effects of Light at Night on the Circadian System of Nurses Meeting; 2005 Nov 16; Boston. Chair: Eva S. Schernhammer, MD, PhD.

2006 Evans EE, Schernhammer ES, Silver ES, Stevens RG, Lockley SW. Reproductive and hormonal risk factors for breast cancer in blind women. Division of Sleep Medicine Annual Poster Session, Harvard Medical School; 2005; Jun 13; Boston

## CONCLUSIONS

In summary, while no conclusions can be drawn at this time between breast cancer risk and visual impairment, there are preliminary data suggesting alterations in risk factors such as BMI, alcohol intake, and onset of menopause compared to the national population, that support a reduced risk of breast cancer for visually impaired women. Data collection is ongoing and we will conduct formal analyses on the full cohort in due course.

Confirmation of the inverse relationship between visual impairment and breast cancer risk and the identification of factors that account for the lower risk of breast cancer in blind women may result in health advice or therapies applicable to blind and sighted populations. Characterization of the potential role of endogenous melatonin rhythmicity in breast cancer risk is a required step towards clinical trials of melatonin administration as a treatment or preventative measure for breast cancer.

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**APPENDIX A**

**Reproductive and hormonal risk factors for breast cancer in blind women**
**Summer undergraduate program 2005**

**Division of Sleep Medicine**
**Brigham and Women's Hospital**
**Boston, MA**

Steven W. Lockley, Ph.D. and Erin E. Evans, R.PSG.T

The summer undergraduate internship program in 'Reproductive and hormonal risk factors for breast cancer in blind women' will be held in the Division of Sleep Medicine (DSM), Brigham and Women's Hospital, Boston.

| | | |
|---|---|---|
| June 1 | 12:30-14:00 | Welcome lunch |
| June 6 | 12:30-14:00 | Introduction to circadian rhythms and melatonin [SWL] |
| June 7 | 15:30-17:00 | Introduction to epidemiology and the Nurses Health Study [ESS] |
| June 14 | 15:30-17:30 | Breast cancer advocacy [ES] |
| June 22 | 14:00-16:00 | Breast tumor biology [KSA] |
| June 28 | 15:30-17:30 | Breast cancer etiology and treatment [WYC] |
| July 12 | 15:30-17:30 | Biochemistry of breast cancer [DEB] |
| July 19 | 15:30-17:30 | Hill's criteria [RGS] |
| July 26 | 15:30-17:30 | Circadian photoreception [SWL] |
| Aug 2 | 15:30-17:30 | Scientific communication [RGS] |
| Aug 9 | 15:30-17:30 | Chronobiology of breast cancer [WJMH and PAW] |
| Aug 16 | 15:30-17:30 | Medical Chronobiology [SWL] |

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